

Blood Donor Screening Process and Infectious Disease Testing Using Molecular Methods

By Barbara Kraj, MS, CLS(NCA), MT(ASCP);
and Teresa Nadder, PhD, CLS(NCA), MT(ASCP)

Molecular diagnostics for screening infectious agents in donor blood is not used to its full potential. The most cited reasons are lack of cost-effectiveness and desirable automation level.¹

To date, several nucleic acid tests (NATs) are FDA-approved for screening donor blood in an effort to diminish transmission of transfusion-related infectious agents, specifically transmissions occurring due to donations collected

during the preseroconversion window when serological tests cannot detect the host's immune response.

Manufacturers have developed NAT assays to detect human immunodeficiency virus type 1 (HIV-1), hepatitis C virus (HCV), hepatitis B virus (HBV) and West Nile Virus (WNV).

HIV-1

The AIDS pandemic has prompted increased alertness from the U.S. Congress and government agencies, as well as blood banks and blood bank product manufacturers, about America's blood supply safety regarding infectious disease transmission potential.

It comes as no surprise the first FDA-approved molecular diagnostic test was developed to detect HIV RNA through reverse transcriptase PCR (RT-PCR) in 2001.

As stated by the FDA Approval Information Notice, the UltraQual HIV-1 RT-PCR assay developed by the National Genetics Institute (NGI) was approved for "qualitative detection of HIV-1 of ribonucleic acid (RNA) in pooled human source plasma comprised of equal aliquots of not more than 512 individual plasma samples."²

The assay was to be used "as an alternative to the licensed HIV-1 p24 antigen tests

for screening source plasma" and was not to be distributed but performed by the NGI at a specified location. According to the Summary of Basis for Approval, the method's specificity and sensitivity exceeded those established for HIV-1 p24 antigen tests.³

In December 2003, Roche Molecular Diagnostics developed the COBAS AmpliScreen HIV-1 test, intended for "testing for source plasma donors in a mini-pool format of up to 96 donations with single unit resolution testing; and testing individual plasma samples from other living donors and organ donors (when specimens are obtained while the donor's heart is still beating)," as stated in the approval notice.⁴

The technology was also based on RT-PCR; however, the post-PCR manipulations were dramatically reduced compared with lengthy and manual techniques employed by UltraQual HIV-1 test, as product detection was performed by spectrophotometry within the same instrument performing the amplification.⁴

Not requiring electrophoresis and subsequent transfer onto a membrane for appropriate band staining was a tremendous advantage of COBAS AmpliScreen test. Also, with technology provided by Roche, the testing could be performed in multiple locations throughout the U.S., not just at the original lab where it was developed.

HCV

In a 1999 statement before the FDA's Blood Products Advisory Committee (BPAC) regarding pooled NAT, AABB said, "pooled NAT for HCV will offer the greatest reduction in the number of potentially infectious units. Such testing could reduce the window period for HCV from the current 70-80 days to an estimated 10-30 days, thereby reducing the per unit risk of HCV transmission from the current estimate of 1:100,000 to 1:500,000-1:1,000,000."⁵

Based on the same technology as the NGI HIV-1 assay, the UltraQual HCV RT-PCR assay received FDA approval concurrently with the HIV-1 test.

Again, as in the case of HIV-1, a much more technologically advanced COBAS AmpliScreen HCV assay was approved in May 2004. Meanwhile, Gen-Probe Inc. developed a Procleix assay based on its transcription mediated ▶

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Upon completion of this article, the participant should be able to:

1. identify several NAT tests to identify infectious disease;
2. distinguish the reasoning behind introducing NAT into donor blood screening; and
3. identify some ethical and legal considerations governing blood supply agencies.



amplification/hybridization protection assay (TMA/HPA) technology, initially approved by the FDA to concurrently detect both HIV-1 and HCV RNA in “human plasma from donations of whole blood and blood components for transfusion” and later for “testing for source plasma donors in a mini-pool format of up to 16 donations with single unit resolution testing; and testing individual plasma specimens from other living donors and organ donors (when specimens are obtained while the donor’s heart is still beating) and for testing individual cadaveric specimens (obtained from non-heart-beating donors)” as stated in extended use notice dated June 4, 2004.⁶

WNV

Since November 2002, AABB has issued multiple statements regarding WNV prompted by the reports of confirmed transfusion transmission and subsequent death due to this infectious agent. In a 2003 statement, AABB stressed “blood donor screening by minipool NAT will be the most significant intervention to decrease WNV transfusion risk.”¹² In December 2005, the Gen-Probe TMA/HPA based Procleix WNV assay was FDA approved and intended for “qualitative detection of WNV RNA in plasma specimens from individual human donors, including volunteer donors of whole blood and blood components, and other living donors;

by researchers from the Heart, Lung and Blood Institute at NIH and from ARC point to real-time reverse transcriptase quantitative PCR.^{15,16} Developing detection assays is paralleled by investigating effective B-19 inactivation treatments which could eliminate the need for NAT.¹⁷

HAV

The FDA and AABB position on introducing hepatitis A virus (HAV) NAT is similar to their position on parvovirus B-19. The June 18, 2000, AABB statement before BPAC supports pool HAV NAT performance as in-process manufacturing control due to low HAV transmission risk.¹⁸

In 2005, a World Health Organization International Standard for HAV RNA nucleic acid amplification technology assays was published by J. Saldanha from Roche, and a collaborative group from 16 laboratories from 10 countries.¹⁹

In a 2003 statement, AABB stressed ‘blood donor screening by minipool NAT will be the most significant intervention to decrease WNV transfusion risk.’

Gen-Probe went further in assay development by including oligonucleotides homologous to HBV-specific sequences to simultaneously detect three infectious agents in pooled samples. However, this Procleix Ultrio assay has not been FDA approved for HBV donor blood screening.⁷

HBV

On March 15, 2001, AABB issued a statement on HBV NAT versus HBsAg, explaining AABB would not give priority to NAT over HBsAg testing unless greater sensitivity was shown.⁸

The only FDA-approved NAT assay in 2005 for HBV was the Roche COBAS AmpliScreen HBV test intended as a “qualitative in vitro test for the direct detection of HBV DNA in human plasma from donations of whole blood and blood components for transfusion, and source plasma, as well as for testing individual plasma samples from other living donors and organ donors (when specimens are obtained while the donor’s heart is still beating).”⁹

In a pre-approval AABB statement issued in 2004, concerns regarding the little benefit and high cost of minipool (24 maximum) HBV NAT was raised again based on two articles published in *Transfusion*.^{10,11} According to the FDA approval letter for HBV NAT, the use of this assay remains optional and is not to replace Anti-HBc.¹¹

testing plasma specimens to screen organ donors when specimens are obtained while the donor’s heart is still beating, and testing blood specimens to screen cadaveric (non-heart-beating) donors.”¹³

Parvovirus B-19

On Dec. 12, 2002, the AABB, America’s Blood Centers (ABC) and the American Red Cross (ARC) presented a joint statement to the FDA BPAC, recommending phase 1 parvovirus pool NAT testing be performed by blood centers as an in-process manufacturing control during plasma-derived product creation, including Factor VIII concentrates which had been previously linked to parvovirus B-19 transmission.¹⁴

Phase 1 testing is not considered true donor testing, does not include the positive pool result to be investigated to the individual donor level, and does not require donor notification or donor exclusion because the self-limiting infection resolves before the donor is eligible for another donation.

At the time the statement was issued, a disclaimer noted the possibility of phase 2 existed. However, due to low risk of serious infection in recipients, this was not a priority. The document does not specify whether the implemented NAT would be PCR- or TMA-based. Two recent studies published

HTLV I and II

During an FDA workshop, “Behavior-Based Donor Deferrals in the NAT Era,” Human T cell lymphoma leukemia virus (HTLV) I and II transmission via transfusion was discussed.²⁰ The viruses’ prevalence coincides with locations shown to exhibit high injection drug use (e.g., United States and Europe). HTLV-I contributes to T-cell leukemia development, and both viruses are responsible for HTLV-associated myelopathy and tropical spastic paraplegia (HAM/TSP), as well as other diseases. Currently, testing for HTLV is performed by EIA screening assays.

These assays’ sensitivity was brought into question during the workshop and presenters expressed the need to develop HTLV NAT. The FDA’s reasoning for not considering HTLV NAT might involve the high incidence of false-positive rather than false-negative results. Although this means there is no risk to potential recipients, blood inventory is deprived of false-positive units.

In 1995, researchers from Retrovirus Study Group of the French Society of Blood Transfusion showed out of 174 samples considered indeterminate by Western Blot (following repeatedly reactive EIA), only three were truly positive by PCR using HTLV specific primers.²¹ However, interpreting this older study is somewhat



complicated, as HTLVs are predominately cell-associated viruses and it is not known if serum testing results reflect infectivity potential. Perhaps leukoreduction and storage conditions are sufficient to prevent transmission, another reason not to focus on HTLV NAT development.

HHV-8

Human herpesvirus 8 (HHV-8), another cell-associated virus, was also discussed during the workshop on behavior-based donor deferrals.²⁰ The virus is an agent of Kaposi's sarcoma and usually co-transmits with HIV. Leukoreduction seems to be an efficient preventive measure against transmission. Interestingly, the presenters stated a great deal of PCR testing was attempted for HHV-8 at CDC, but thought "NAT would not be practical due to low viral loads."²⁰

This position is also supported by a publication of the retrovirus epidemiology donor study. Its purpose was to evaluate the usefulness of HHV-8 PCR by six U.S. laboratories that unsuccessfully tried to amplify viral DNA in the same set of donors.²²

CMV

Cytomegalovirus (CMV) NAT could be used to replace current serological testing to identify CMV-safe units. The attempts to develop an efficient molecular assay have been undertaken by J.D. Roback and others in the Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta; and by a laboratory led by Michael Boeckh, MD, from Fred Hutchinson Cancer Research Center and the University of Washington at Seattle.²³

The first group using the Roche Monitor assay and nested PCR directed at the CMV UL93 open-reading frame did not see an increased detection of CMV as compared to serologic screening assays.²⁴

Dr. Boeckh's group claims a double primer TaqMan real-time PCR assay developed in their lab is capable of CMV DNA detection in plasma before seroconversion.²⁵ The FDA is not currently considering the assay to be introduced in donor screening. Because the study was performed on a population of hematopoietic stem cell transplant (HSCT) recipients, it would have to be repeated in blood donors first.

Treponema Pallidum

On June 16, 2000, AABB issued a statement before the FDA BPAC recommending STS be eliminated from whole blood donation testing.²⁶

The recommendation was based on an Arcnet Epidemiology Group presentation during the 1999 AABB meeting citing evidence for the lack of *Treponema pallidum* DNA in FTA-ABS confirmed positive platelets. These findings were published in 2002.²⁷

Two different PCR assays (single and multiplex) were targeted at two different TP genes as well as an RT-PCR assay; all failed to demonstrate any presence of DNA and RNA. The conclusion was no infectious agents were present in FTA-ABS positive samples.

The fact the negative molecular test result prompted this conclusion rather than the suspicion of questionable sensitivity of the test itself was supported by the fact for 30 years no transfusion-transmitted syphilis was observed.

The recommendation to eliminate syphilis testing was repeated later in 2000 with an accompanying statement declaring AABB's support for FDA's further research of the subject. In 2003, FDA published guidelines on syphilis testing which AABB had addressed as being impractical.²⁸

Seroconversion Window

According to the Report of the Interorganizational Task Force on Nucleic Acid Amplification Testing of Blood Donors in 2000 and Kolk, et al., blood donations collected during the preseroconversion window are the main cause for HIV, HCV and HBV transfusion-related transmission.^{29,30}

The reported seroconversion window lengths for each infectious agent vary among the researchers. In case of HIV, the task force reports a median of 40 days from exposure to seroconversion, while Kolk and others report 22 days. Both sources admit in some cases, seroconversion may take as long as 6 months. Seroconversion lengths may be difficult to establish due to the eclipse period immediately following the exposure, when the virus replicates within the lymph nodes and is not released into bloodstream.

NATs for HIV-1 and for HCV have

had the biggest impact on blood supply safety due to significant window period reduction. The FDA Draft Guidance for Industry on Nucleic Acid Testing for HIV and HCV provides recommendations based on at least two reviews on infectious window period.³¹⁻³³

Because the residual transmission risk is calculated by multiplying the incidence rate by the number of days during which the infection can occur, the reduction of the window period length decreases the residual risk of infectious disease transmission.

As reported by both authors cited in the FDA document, the average seroconversion window period for HIV is 11 days (compared with 22 days when using serological testing) and the average seroconversion window period for HCV is 10 days (as compared to the original 82 days when using serological testing).

Consequently, due to NAT implementation, the residual risk of HIV transmission decreased to one incident per 2,135,000 donations, and the residual risk of HCV transmission decreased to one incident per 1,935,000 donations. These data certainly provide enough evidence for the benefits of NAT introduction into donor blood screening to increase America's blood supply safety.

NAT's Propelled Intro

The single most obvious factor propelling NAT into donor blood screening was the rapid development of molecular methods following the discovery of PCR technology by Kary Mullis in the mid-1980s. Though this technology was quickly introduced into basic research laboratories, several obstacles needed to be overcome for clinical applications.

Much manual manipulation following the PCR, the laborious gel castings, staining using carcinogenic agent and UV-light photography prohibited molecular tests from easy access into clinical diagnostics. Lacking molecular diagnostics training among medical technologists has also contributed to the delay.

However, due to obvious advantages resulting from the seroconversion window reduction, both DHHS and FDA encouraged NAT introduction into the donor blood screening process. Also, in 1997, the European Committee for Proprietary ▶



Medicinal Products required, as of July 1999, all fractionated plasma products be tested for HCV using NAT assays. This has contributed significantly to NAT implementation in the U.S. because many blood banks exported blood and blood products to Europe.³⁴ The 1999 implementation of NAT screening in Europe was triggered by the HCV infections resulting from IV administration of immunoglobulins.

Current Issues

The main driving force for implementing NAT in American blood banks should be its contribution to the “zero-risk blood supply” initiative. The FDA calls NAT testing “the most sensitive method currently available for detection of blood-borne viruses.”³⁵

Despite the positive political atmosphere resulting from the governing bodies’ perception of infectious disease dangers, many issues regarding NAT remain under discussion. As mentioned previously, automation deficiencies prohibit routine single donor testing in the near future.

Some expectations are connected with microarray technology; however, these are not presently adjusted to blood banking needs. The GreeneChip technology (mentioned in an issue of *AABB News*, presently under development in the Greene Infectious Disease Laboratory at Columbia University), although high-throughput, has a sensitivity lower than standard PCR.³⁶ The BeadChip technology owned by BioArray Solutions Inc. has not been really considered for infectious disease testing, although molecular RBC antigen typing attempts have been made.³⁷ The desired automation points to another issue in NAT implementation: cost prohibition. NAT implementation costs are estimated at about 96 million dollars per year.¹

Costs include not only equipment and reagents, but also adjusting blood bank operations to new technology which, due to contamination potential, require lab and workflow redesign. Personnel training costs should not be overlooked either.

In 2000, the Task Force of Nucleic Acid Testing in Blood Donors divided NAT issues into six categories:

1. general (availability, potential use, education);

2. test-related (standardization, definition of positivity, sensitivity, licensure);
3. donor-related (consent, in view of IND status at the time);
4. positive donor management;
5. hospital-related (positive sample management); and
6. recipient-related (follow-up and counseling).

Due to nucleic acids unique character as genetic information carriers, DNA samples and sequence data should be protected from personal privacy abuse.³⁸

Long before NAT implementation for donor screening, the Ad Hoc Committee on DNA Technology, DNA Banking and DNA Analysis of the American Society of Human Genetics raised several questions regarding molecular diagnostics, including:

- Under what circumstances, if any, should the DNA diagnostic laboratory release results to anyone other than the patient?
- Under what circumstances, if any, should the DNA bank or laboratory transfer deposited DNA to a party other than the patient?
- What is the responsibility of the DNA diagnostic laboratory for reported result accuracy?
- Under what circumstances is it permissible to use deposited DNA for purposes unrelated to the original request of the depositor?³⁹

The potential answers to these questions formulated years ago may have serious legal repercussions and should be addressed in documents released by blood supply governing agencies.

Pooled Testing

While introducing NAT, practicality was the most straightforward advantage of pooled samples testing. In the absence of high throughput automated technologies, pooled testing offered a good compromise.

Pooled testing also seemed reasonable due to the high sensitivity of PCR and TMA amplification methods, able to detect a single gene copy (single cell PCR) in basic research labs. In terms of sensitivity, this means 5000 IU of RNA in case of HCV, consistent with high dilutions.⁴⁰

Such high sensitivity was a best-case scenario, proven incorrect more frequently than anticipated. Pool ultra-centrifugation concentrated the viral particles and

prevented the dilution effect. This method, however, was not effective in case of lipid-bound HCV particles.⁴¹

In addition to dilution effect, the presence of DNA or RNA polymerase inhibitors in one donor sample may compromise other samples in the pool, especially when the pool size is small.

The Clinical and Laboratory Standards Institute lists several contributors to false-negative results due to inhibition.⁴² According to a report from the Interorganizational Task Force for NAT, “negative NAT results on a single donor are more specific than a reactive result on a pooled sample.” On Sept. 12, 2002, AABB issued a statement on single donor NAT recommending a transition from pooled to single donor NAT with accompanying automation technology.⁴³ ■

Barbara Kraj is assistant professor, department of biomedical and radiological technologies, Medical College of Georgia at Augusta. Dr. Nadder is associate professor and department chair, Virginia Commonwealth University at Richmond.

References

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